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**EFFECT OF PROTEIN-CALORIE MALNUTRITION ON FOOD CONSUMPTION,
WEIGHT GAIN, SERUM PROTEINS, AND ACTIVITY IN THE DEVELOPING
RHESUS MONKEY (MACACA MULATTA)**

By

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B.S., University of San Diego, 1968

Presented in partial fulfillment of the requirements for the degree of

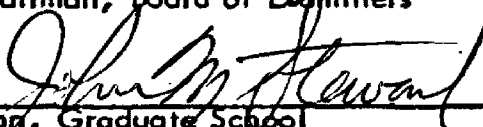
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EFFECT OF PROTEIN-CALORIE MALNUTRITION ON FOOD CONSUMPTION, WEIGHT GAIN, SERUM PROTEINS, AND ACTIVITY IN THE DEVELOPING RHESUS MONKEY (*MACACA MULATTA*)^{1,2,3}

CHARLES R. GEIST, ROBERT R. ZIMMERMANN, AND DAVID A. STROBEL

SUMMARY • Five groups of infant rhesus macaques were separated from their mothers at 90 days of age, housed in individual cages, and placed on purified diets that were isocaloric but contained either 25% (high), or 2% or 3.5% (low) protein by weight at 380, 210, or 120 days of age. The subjects sustained on the low protein diets showed a marked reduction in weight gain when compared to high protein controls. On the whole, monkeys on diets deficient in protein consumed less than animals fed normal quantities of protein. However, all groups, regardless of diet regime, consumed food in quantities that were proportional to their body weights. Analysis of blood serum components revealed values of albumin and total protein which consistently reflected the level of dietary protein fed. Globulin serum levels, however, consistently failed to relate to the dietary level of protein. Activity, as measured in a living cage situation, showed no effects of diet and suggested that the low protein animal had a general level of activity that was equal to that of the high protein control.

In recent years, interest arose concerning the effects of protein-calorie malnutrition on the behavior and development of man (1-3) and animals (4-6). The term protein-calorie malnutrition was first introduced by Jelliffe (7) and later was proposed by the Joint Food and Agriculture Organization and the World Health Organization Expert Commission on Nutrition (8) in reference to a diversity of clinical deficiency diseases. Attention, however, was focused primarily on kwashiorkor (9, 10) or on the kwashiorkor-like syndromes (11, 12).

A number of biochemical, clinical, and behavioral factors are manifest in calorie and protein deficiencies and have been under

investigation by a variety of researchers (13-18). Barnes *et al* (19, 20) and Levitsky and Barnes (5) found that food intake when equated on the basis of body weight was greater in previously malnourished male rats, but not female rats. Weight gain in infant rhesus monkeys was shown to increase rapidly on diets containing high or standard protein concentrations while low protein animals maintained their weight (21). Blood serum protein changes included a decrease in total serum protein, albumin, and albumin/globulin ratio for protein deficient human infants (3) and rhesus monkeys (21). Activity, as a function of low protein diets, increased in rats as measured in an activity wheel (22, 23) and has been corroborated on other activity measures with previously malnourished rats and pigs (5).

This investigation was designed to delineate 4 parameters arising from diets deficient in

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protein which were fed to developing rhesus monkeys (*Macaca mulatta*). These factors included food consumption, weight gain, serum protein, and activity. All of these measures have previously been shown to be valuable indicators of the biochemical, clinical, and behavioral concomitants of protein-calorie malnutrition. The diets were isocaloric, but the protein concentration was manipulated to yield diets containing either 25%, 3.5%, or 2% casein by weight. Our primary concerns, therefore, were to: 1) determine the *ad libitum* food consumption value as a function of dietary protein deficiency in developing rhesus monkeys equated with respect to age and size, 2) relate the food consumption figures with weight gain in protein malnourished animals, 3) evaluate the effects of these diets on serum protein, albumin, and globulin, 4) ascertain whether activity as a behavioral measure varies with increasing dietary protein restriction.

MATERIALS AND METHODS

Experimental groups: Five groups of infant rhesus macaques were separated from their mothers at 90 days of age and maintained on the milk formula diet described by Blomquist and Harlow (24). Procedures identical to those set forth by Zimmermann (25) for the introduction and weaning to solid food were employed, and at specified intervals the groups were placed on the experimental diets (Table 1). These diets were isocaloric but contained either 25% (high) protein, or 2% or 3.5% (low) protein by weight. Groups 1 (N=4), 2 (N=6), and 4 (N=5) were introduced to the 3.5% low protein diet at approximately 380, 210, and 120 days of age, respectively. At 1,036, 728, and 500 days, groups 1, 2, and 4 were shifted to a 2% low protein diet in order to maintain a stable body weight. Control groups 3 (N=4) and 5 (N=4) for animals in groups 2 and 4 continued on the 25% high protein at 210 and 120 days of age, respectively.

TABLE 1

Composition of experimental diets

Components	Low pro- tein 2%	Low pro- tein 3.5%	High pro- tein 25%
Primex 1/	9.0	9.0	9.0
Fat-soluble vitamin	1.0	1.0	1.0
Crude casein	2.0	3.5	25.0
Cerelose 2/	39.7	39.0	29.0
Dextrin 3/	39.9	39.2	27.7
Salts (HMW) 4/	4.0	4.0	4.0
B-vitamin premix	2.0	2.0	2.0
Choline dihydrogen citrate	0.3	0.3	0.3
Ascorbic acid	0.03	0.03	0.03
Alphacel 5/	2.0	2.0	2.0
	99.93	100.03	100.03

¹Primex, Procter and Gamble Co, Cincinnati, Ohio

²Cerelose, Coin Products Co, Argo, Ill.

³Dextrin (white tech), Nutritional Biochemicals Corp, Cleveland, Ohio

⁴Hubbell RB, Mendel LB, Wakeman AJ, Salt Mixture, Nutritional Biochemicals Corp, Cleveland, Ohio

⁵Alphacel (non-nutritive bulk), Nutritional Biochemicals Corp, Cleveland, Ohio

B vitamins in 2 grams cerelose	mg
Thiamine HCl	0.40
Riboflavin	0.80
Pyridoxine HCl	0.40
Ca pantothenate	4.00
Niacin	4.00
Inositol	20.00
Biotin	0.02
Folic acid	0.20
Vitamin B-12	0.003
Menadione	1.00
Fat-soluble vitamins in corn oil	mg
Vitamin A acetate	0.31
Vitamin D (calciferol)	0.0045
Alpha-tocopherol	5.00

Subjects in group 1 served as pilot animals which were placed on restricted diets in order to establish that rhesus monkeys could be satisfactorily maintained at low levels of protein deficiency without mortality. For convenience, each group is referred to by the mean age of the group at the onset of either the 25% or 3.5% protein diets.

Food preparation: The diets were prepared by adding each of the ingredients to

an 80 quart Hobart mixer (Hobart Co, Helena, Mont) and blended until a dry homogeneous powder was obtained. To differentiate each of the diets with respect to protein concentration, a sufficient quantity of either red or blue food coloring was suspended in the fat-soluble vitamin mixture prior to blending, coloring the diets as follows: 25% (high) protein—blue, 3.5% (low) protein—yellow, and 2% (low) protein—red. The diets were then refrigerated until needed. From the dry dietary preparations biscuits were formed weighing from 150–200 g each prior to feeding the animals. To accomplish this, water just sufficient to make a dough was added to the diets. The dough was then rolled out on a cutting board and individual biscuits were fashioned by the use of a circular cookie-cutter approximately 2" in diameter. Each of the animals was fed *ad libitum* quantities of their respective diets.

Food consumption: For 30 consecutive days the quantity of food consumed was determined for the 380, 210, and 120 day low protein groups at 969, 786, and 1,018 days of age, respectively, and for the 210 day high protein group at 745 days of age. Subjects were housed in 24" (l) x 24" (h) x 24" (w) individual cages of a design that provided moderate protection against food spillage but did not sacrifice visibility or cleanliness. The animals were fed a measured 150–200 g of diet in biscuit form every evening at 5:00 pm. This amount had previously been found to be greater than the maximum dietary intake that each animal would consume in 24 hr. The following morning the drop-pans from each of the cages were removed and the food residue separated from the fecal matter. The collected food was air dried and weighed. The weight of the food fed the animals minus the residue was taken as the estimate of food consumption. The animals could reach through the cage floor to the pan in order to retrieve solid food that spilled through the wire mesh, thereby increasing the accuracy of this measure.

Weight gain: The weighing procedure in-

involved training all animals to enter an aluminum transport cage containing a sliding door that was placed directly in front of the permanent cage door. After several training sessions in which animals were forced into the transport cage, all subjects entered the cage voluntarily. Thus, no manual handling was required in this procedure. Once trained in this manner, a tared transport cage, designated solely for weighing, was utilized to determine the weights on every animal each morning prior to feeding and daily testing activities. The transport cage was cleaned when necessary between weighings. Weights were determined on an Ohaus Heavy-Duty Solution Balance (Ohaus Co, Union, NJ).

Serum protein, albumin, globulin, and albumin/globulin ratio: At least 2 cc of whole blood was drawn from the femoral or saphenous vein of restrained monkeys with a 23 ga, 1" needle, on a 2½ cc heparinized syringe. The serum was isolated by centrifugation at 1500–2000 rpm for 10 min in a Sorvall, Model SS-4 centrifuge (Sorvall, Inc, Norwalk, Conn). An aliquot was then subjected to electrophoretic analysis at a clinical laboratory (Saint Patrick's Hospital, Missoula, Mont) employing cellulose acetate strips in phosphate buffer at pH 8.6 in a Gelman Migration Chamber (Gelman Instrument Co, Ann Arbor, Mich) and a Photovolt Densicord Scanner and Integrator, Model 9 (Photovolt Corp, New York, NY). Electrophoretic values for albumin, globulin, and total protein were determined annually for 2 yr. Periodic total protein determinations were conducted in our laboratory on all groups at 6-month intervals by the colorimetric biuret method of Weichelbaum (26), as described by Hiller (27), in a Leitz Photometer (E. Leitz, Inc, New York, NY). Standard control serum was used as a reference for comparison of values (Hyland Laboratories, Los Angeles, Calif).

Activity: Activity was measured as a general movement within a cage for all subjects throughout the dietary regime. Movement was detected by 2 photocells, each mounted

15" from the floor of a cage approximately 30" (l) x 30" (w) x 30" (h). The subjects were placed singly in the cage for 1 hr per day, approximately every third day, allowing time to similarly test other animals in the colony. In order to balance out daily cyclic factors, the time of day the animals were placed in the apparatus varied between the hours of 6:00 am and 5:00 pm. The cage was located in a 6' (l) x 8' (w) x 8' (h) room that was empty except for a 38" (l) x 17" (w) x 31" (h) box that contained recording equipment. The box was lined with insulation material to attenuate electromechanical noise from relays and counters. The room was lighted by a 30 watt overhead light and a 15 watt signal lamp located on the side of the recording apparatus. Inside the cage a series of bars were mounted across the floor to a height of 10" to insure that the animals pacing about the cage would break either of the photobeams. The apparatus was designed to approximate the living cage in size and shape. Subjects were not tested in the same rooms as their living quarters in order to reduce variability in activity records resulting from disturbances produced either by laboratory personnel during the normal execution of their duties, or distractions produced by the presence of other monkeys. These data report activity measured in this device for a period of 2 yr.

RESULTS

Developing rhesus macaques, equated with respect to age and size, show little difference in food consumption across the dietary regimes. As a whole, the monkeys on diets deficient in protein consumed less food than either those fed standard or elevated protein concentrations. Furthermore, the absolute quantities of the diets consumed within the protein deficient groups appeared to be ordered by age, with the older animals eating a larger amount of food. The ratio of food consumed/g body weight was used to determine if any intergroup variations existed

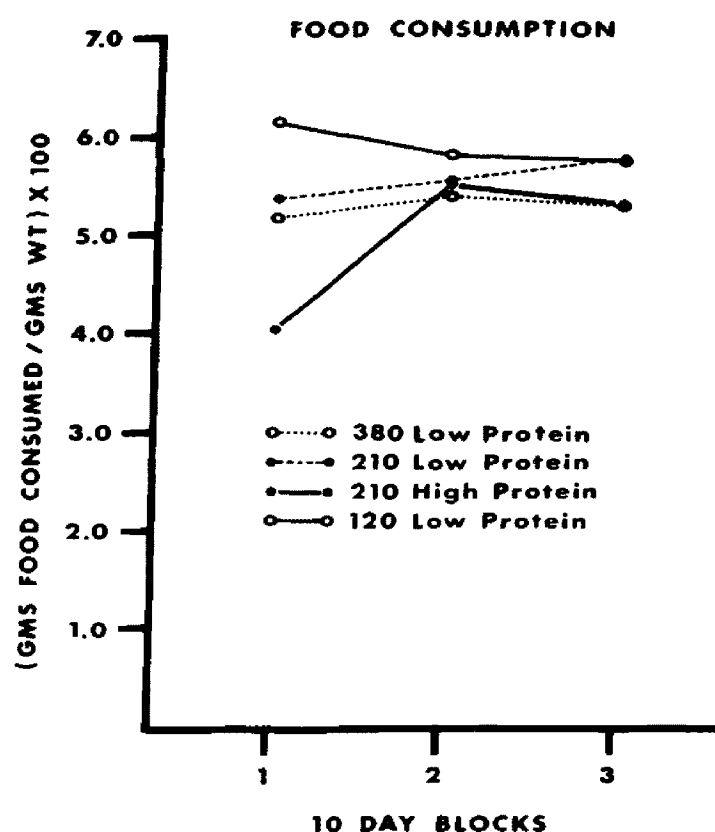


Fig 1. Food consumption ratio: mean grams food consumed per grams body weight for the 380, 210, and 120-day low protein groups and the 210-day high protein group.

when the absolute consumption of food was related to body weight. No significant differences were found. There was a considerable overlap among groups, indicating that the monkeys, independent of the protein concentration contained in the diet, consumed a quantity of food that was proportional to their body weights (Fig 1).

The diets deficient in protein produced a marked effect on body weight gain. The mean body weights over 800 days in 50-day blocks for the 380- and 210-day low protein groups, and the age equivalent 210-day control group are illustrated in Fig 2. The 380-day low protein animals showed less than a 15% weight gain over a 3-yr period. Similarly, the 210-day low protein group had only a 25% gain over a 2½-yr period, while the 210-day high protein control group made nearly a three-fold increase in body weight

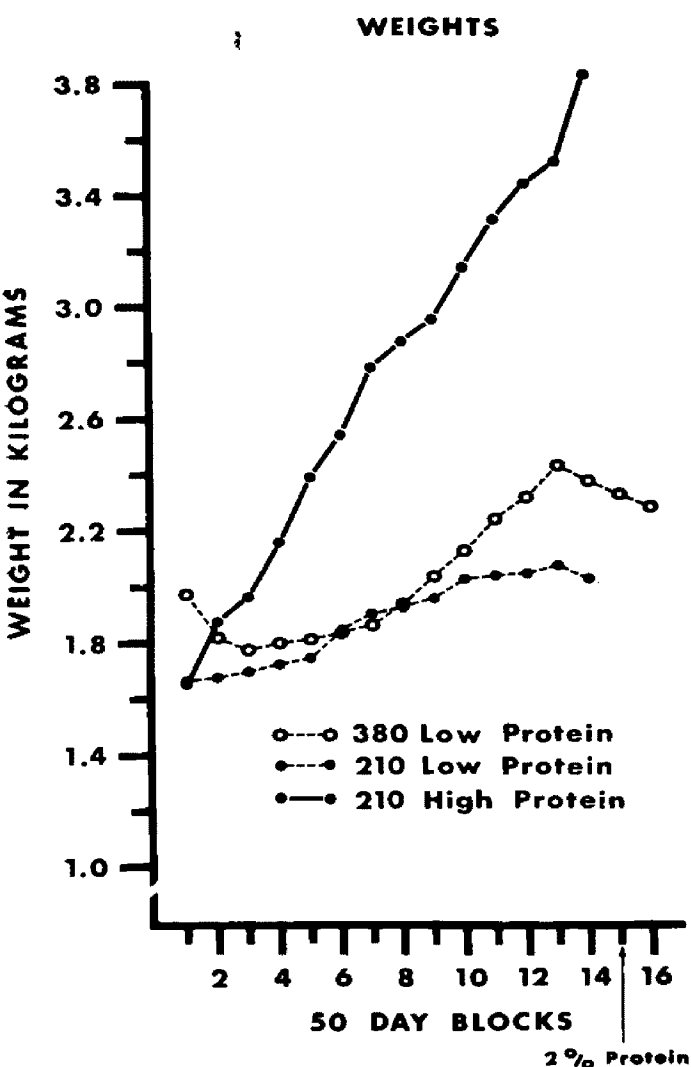


Fig 2. Mean body weights over 800 days in 50-day blocks for the 380- and 210-day low protein groups and the 210-day high protein group from the onset of diets.

over the same period. Depicted in Fig 3 are the mean body weights for the 120-day low protein group and its age equivalent 120-day high protein control over a period of 200 days in 10-day blocks. The 210-day high protein group gained 4 times the weight which was gained by the 120-day low protein group during the initial 120-day diet period. Each of the 120-day groups were fed a standard human milk formula, Prosobee® (Mead Johnson, Evansville, Ind), and the high protein diet following separation from their mothers. During this initial period the weight gain was similar within these groups, the

diverging values beginning only after the introduction of the experimental diets.

Protein electrophoretic values were determined at 12-mo intervals over a period of 2 yr. Thirty days prior to the second analysis, the 380, 210, and 120 day low protein groups were shifted from 3.5% to 2% protein by weight. The mean electrophoretic values and standard errors for each group obtained in both years are presented in Table 2.

The first year analysis was conducted on the 380- and 210-day low protein groups at 696 and 358 days of age, respectively, and on the 210-day high protein group at 424 days of age. The total protein values were markedly and significantly different (analysis of variance) between the low protein groups and the high protein group. No significant differences were detected between the low protein groups. Similarly, the albumin values were significantly different (analy-

Fig 3. Mean body weights over 200 days in 10-day blocks for the 120-day high and low protein groups before and after onset of diets.

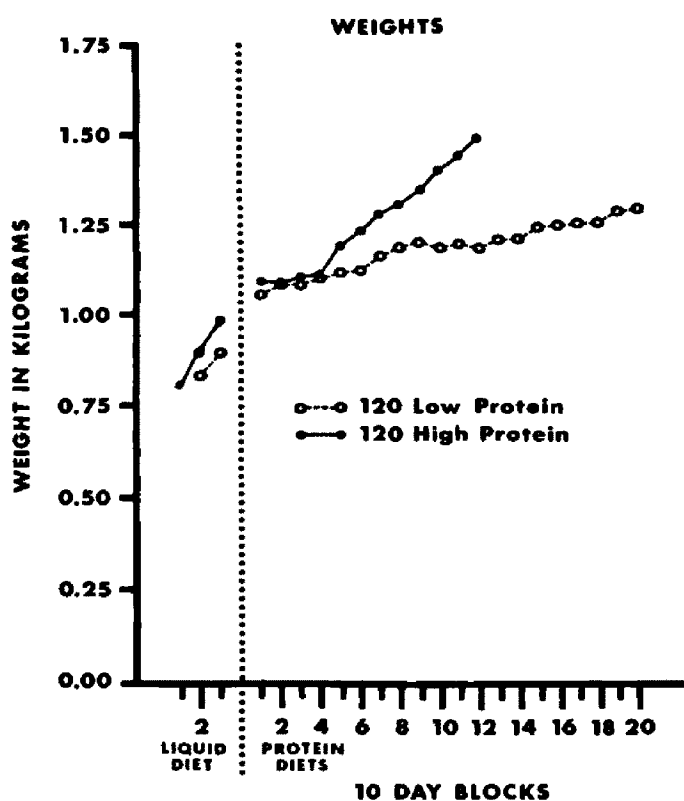


TABLE 2

Mean electrophoretic values of blood serum components

Diet	Days of age at onset of diet	Days of age at collection	Number of subjects	Total protein, g/100 ml \pm SE	Albumin g/100 ml \pm SE	Globulin g/100 ml \pm SE
Analysis 1						
3.5% protein	380	696	4	6.5 \pm 0.5 ^a	4.0 \pm 0.4 ^c	2.4 \pm 0.5
3.5% protein	210	358	6	6.6 \pm 0.3 ^a	3.9 \pm 0.3 ^c	2.6 \pm 0.3
25% protein	210	424	4	7.4 \pm 0.1 ^b	4.8 \pm 0.2 ^d	2.6 \pm 0.2
a,b,c,d Means with the same letter did not differ significantly, $P < 0.01$						
Analysis 2						
2% protein	120	556	4	6.2 \pm 0.4 ^a	3.4 \pm 0.3 ^c	2.8 \pm 0.5
2% protein	380	1106	4	6.2 \pm 0.3 ^a	3.7 \pm 0.4 ^c	2.5 \pm 0.4
2% protein	210	768	6	6.2 \pm 0.3 ^a	3.9 \pm 0.4 ^c	2.3 \pm 0.5
25% protein	210	834	4	7.1 \pm 0.4 ^b	5.0 \pm 0.2 ^d	2.0 \pm 0.4

a,b,c,d Means with the same letter did not differ significantly, $P < 0.01$

sis of variance) between the low protein groups and the high protein group, while there were no significant differences between the low protein groups.

The second yr blood serum assay was conducted on the 380, 210, and 120 day low protein groups at 1106, 768, and 556 days of age, respectively, and on the 210-day high protein group at 834 days of age. Total protein values were significantly different between the high protein group and all low protein groups (analysis of variance). The low protein groups did not differ significantly from one another. Significant differences in albumin values were also found between the high protein subjects and all low protein groups. Again, the low protein groups did not differ significantly from one another. Thus, on both of these measures, the high protein group had significantly higher values than any of the low protein groups. No significant differences were found in globulin values.

Periodic total protein determinations using the biuret method were conducted on all groups. The results were similar to those found in the electrophoretic analysis. Absolute values of total protein were consistently lower, but the intergroup relations still ex-

isted, leading to the same relative significant differences between the groups. A special investigation aimed at discovering the monthly fluctuations in total serum protein was performed on the 380-day low protein group. The monthly mean total serum protein values ranged from a minimum of 6.10 mg/100 ml to a maximum of 6.64 mg/100 ml over a 9-mo period (Fig 4). From these data a standard deviation of 0.18, a mean of 6.43, and a variance of 0.03 were calculated, illustrating a fluctuation within very narrow limits.

Activity records from 125 1-hr sessions were taken of low protein monkeys in the 380, 210, and 120 day groups and on the 210-day high protein group. The fact that there were no significant differences between the groups indicates that for this particular measure low protein monkeys do not appear inferior to high protein monkeys in general activity.

DISCUSSION

The importance of dietary protein for the normal growth and development of man and lower mammals has been stressed by similar

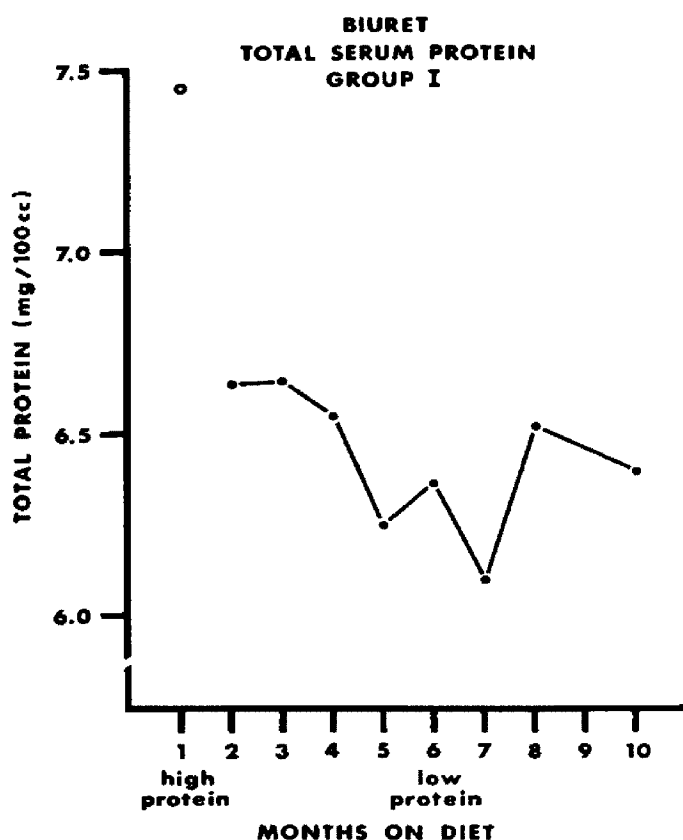


Fig 4. Monthly mean total serum protein concentration (mg/100 cc) for the 380-day low protein group.

observations in clinical and experimental studies (28). Protein-calorie malnutrition has induced a condition of experimental primate kwashiorkor (29). In response to decreased protein intake, retardation of growth and physical development represent the most consistent clinical features (10). In experimental animals the clinical features may include: edema (30); the hair becomes brittle, loses natural lustre, appears thinner, and is easily pulled out (4); weight gain is significantly decreased from normal with the result that protein-deficient animals simply maintain their weight (21); anemia of moderate severity is generally present (29); low body temperature and metabolic rate are marked (31); and decreased levels of serum albumin and cholesterol are observed (32). The results of this investigation indicate that similar effects of protein-calorie malnutrition on the weight gain and serum proteins of developing rhesus macaques can also be produced.

It might be expected that because of their reduced size the malnourished monkeys would consume a quantity of food far less than those animals fed a diet sufficient in protein. In one respect this contention has been confirmed in this investigation. Diets deficient in protein are consumed to a lesser degree than those with adequate protein. This reduced caloric intake could be interpreted as one of the primary sources of the physical retardation of protein-restricted animals; however, this is not necessarily the case. Regardless of the protein concentration of the diet, rhesus macaques were shown to consume an amount of food proportionate to their body weights. The important variable appears to be whether or not the animal can adapt to a diet low in protein and utilize the calories that are available. Waterlow (33) suggested that this adaptation to the diets may take 2 major forms: a decrease in the output of urinary nitrogen and a change in the distribution of protein in the body. His findings indicate alterations in protein synthesis occurring to a reduced degree in muscle and probably the skin, whereas in the liver and perhaps other essential organs it is not. It also appears that the amino acids liberated by the catabolism are more efficiently reutilized for synthesis. Changes in endocrine activity may help to explain the pattern of protein turnover which occurs in adaptation. While it has not been assumed that these represent the only underlying factors peculiar to protein-calorie malnutrition, they lend credence to the need for future research in this area.

The finding that protein-calorie malnutrition results in significantly lower rates of weight gain (2, 10, 19, 21, 29, 31) and decreased levels of total protein and albumin (3, 10, 17, 18, 31) has been confirmed in this investigation. When protein-restricted animals are compared with either high or standard protein animals, there is a marked and significant difference in maximum weight attained, rate of weight gain, and the level at which an asymptote is reached. Blood serum analysis showed significantly lower

levels of total serum protein and albumin for all groups of low protein animals. An analysis of variance revealed that each low protein group when compared with the high protein group differed significantly ($P < 0.01$). No significant differences were noted between any of the low protein groups. These data indicate that the effects of protein-calorie malnutrition on weight gain, total serum protein, and albumin can be reliably reproduced in the developing rhesus monkey.

The effects of protein-calorie malnutrition on the general level of activity in man and experimental animals is little understood and has been seldom investigated. Guthrie (16) investigated activity level in rats in order to determine the extent to which differences in learning might be explained on the basis of motivational or motor factors. It was found that undernourished rats were more active than normal rats. Similarly, Collier, Squibb, and Jackson (22, 23) found that diets deficient in protein produced higher levels of activity in an activity wheel than diets providing adequate protein. The results of the present investigation have shown that activity in rhesus monkeys when measured as general movement around a cage produces no significant differences between any of the low protein groups when compared to the high protein animals. Failure to find activity differences over an extended period and degrees of deprivation despite the clinical manifestations of protein-calorie malnutrition would suggest that malnutrition affects activity selectivity. That is, activity levels may be dependent not only upon the measures used, but also show considerable variation across species.

Studies of malnutrition involving human infants and young children are generally not feasible and often result in confusion due to the interaction of social, economic, and cultural factors. Investigations concerning the effects of protein-calorie malnutrition are more amenable to investigation with the aid of experimental animals under controlled

laboratory conditions. Therefore, the effects of dietary protein restrictions on mental and physical development as well as the conditions necessary for rehabilitation would appear to be more easily investigated in the developing rhesus monkey.

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